cagA Status and Eradication Treatment Outcome of Anti-Helicobacter pylori Triple Therapies in Patients with Nonulcer Dyspepsia

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The differences in eradication rates reported in clinical trials aiming to cure Helicobacter pylori infection cannot be entirely explained by the type of regimen, bacterial resistance, or lack of compliance. Using data from a clinical trial, a logistic regression model was constructed to determine whether cagA status, assessed by PCR, affects the outcome of eradication. Resistance to clarithromycin (10% of the strains) predicted failure perfectly. In the model (n = 156), a cagA-lacking strain (odds ratio [OR] = 2.2; 95% confidence interval [CI], 1.1 to 4.7), tobacco smoking OR = 3.1; 95% CI, 1.3 to 7.0), and a double dose of proton pump inhibitor in the treatment regimen (OR = 0.3; 95% CI, 0.2 to 0.7) were associated with the treatment outcome. The exact role of cagA in the outcome of H. pylori eradication therapy has not been explored. However, the type of histological lesions which it causes in the gastric mucosa may be implicated. Regardless of the mechanism involved, cagA status is a good predictive marker of eradication outcome.

Triple therapies used for the eradication of Helicobacter pylori generally include two antibiotics, i.e., clarithromycin and metronidazole or clarithromycin and amoxicillin, and a proton pump inhibitor. In several European multicenter studies, cure rates from 80 to 95% have been obtained using omeprazole (19), lansoprazole (25), or pantoprazole (11), except in France, where the cure rate varied from 70 to 80% (6).

These large multicenter studies have been performed in northern Europe where compliance is better and where resistance of H. pylori to antibiotics is lower than in Mediterranean countries (23). However, these European studies included exclusively (11, 19) or essentially (25) peptic ulcer disease (PUD) patients, while a large number of patients with nonulcer dyspepsia (NUD) were included in the French studies. Better eradication rates have been reported in PUD patients than in NUD patients, 73 versus 55%, respectively (P = 0.016) (29). A recent meta-analysis also indicated a better efficacy of these triple therapies in PUD patients than in NUD patients (eradication rates of 90.4 and 77.7% respectively [P = 0.001]) (15).

The cagA gene has been found more frequently in strains from PUD patients than in strains from NUD patients (12, 17, 30). The cagA gene is a marker for the cag pathogenicity island, which is associated with an increased inflammatory response at the gastric mucosal level (1, 10) and severe gastric disease (3, 4). Furthermore, the function of the protein produced by this gene has recently been determined by Stein et al. and Covacci et al. (8, 26).

The question of whether to eradicate H. pylori in NUD patients is still debated; therefore, it is interesting to consider the genotype of H. pylori strains when evaluating treatment outcome (5, 22, 27, 28). Although limited by a small sample size, one study has provided promising results on the subject (30). For answering the question, the most practical alternative is to consider clinical trials performed on NUD patients. The main advantages of clinical trials, despite their lack of representativity, are the quality of follow-up, data collection, and methodology.

Therefore, the following analysis was conducted to determine the factors involved in the outcome of eradication treatment, particular the cagA status of the H. pylori strain harbored. The data used came from a large multicenter clinical trial on H. pylori eradication, carried out on NUD patients (18), evaluating a 7-day triple therapy currently recommended in France and Europe (21, 32).

MATERIALS AND METHODS

Study data. The data were issued from a clinical trial carried out by the Aquitaine Gastro Association in southwest France, whose primary aim was to compare two different doses of proton pump inhibitor in a triple therapy for H. pylori eradication.

This multicenter, randomized, double-blind trial was conducted on patients with NUD, confirmed by endoscopy, with or without a history of past ulcers. The two arms of treatment were: amoxicillin (1 g twice a day b.i.d.), clarithromycin (500 mg b.i.d.), and pantoprazole (40 mg once a day o.d.); versus amoxicillin (1 g b.i.d.), clarithromycin (500 mg b.i.d.), and pantoprazole (40 mg b.i.d.). H. pylori status was assessed by PCR, endoscopy, histology, or culture and at 4 weeks after the end of treatment by histology or culture or urea breath test if the patient refused the posttreatment endoscopy.

In this trial, a total of 223 patients were randomized, 192 were included in the intention to treat analysis and finally 166 patients were included in the per protocol (PP) analysis (18). The description of the 37 patients excluded from the trial and the results of the clinical trial have been published (18).

Study population. The present analysis included the PP population of the above-mentioned trial, for whom the cagA gene status of the H. pylori strain was available. This population was chosen because the patients had indeed received a treatment which had or had not been successful. H. pylori culture and resistance tests. Culture of H. pylori was performed on selective and nonselective media (24) before treatment and 4 to 6 weeks after the end of treatment. The MICs of clarithromycin for H. pylori were determined by E-test.
TABLE 1. Nucleotide sequences of primers used to detect the \textit{cagA} gene$^a$

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' to 3')</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>GATAACGAGCCGTTTGGGGA</td>
<td>157-181</td>
</tr>
<tr>
<td>A2</td>
<td>CCATGATTTTATGTCAGTC</td>
<td>550-527</td>
</tr>
<tr>
<td>A3</td>
<td>AGGCTGACGATCGTGGGACCA</td>
<td>910-935</td>
</tr>
<tr>
<td>A4</td>
<td>ATTAGGC621AAGAGCGGCC</td>
<td>1626-1602</td>
</tr>
</tbody>
</table>

$^a$ The indicated primers were used in PCRs consisting of 40 cycles as follows: 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C.

Bacterial suspensions equivalent to a McFarland opacity standard of 3 were prepared in brucella broth from 48-h-old agar plates and were used to flood Wilkins-Chalgren agar plates enriched with 10% human blood without antibiotics. After drying, E-test strips (AB Biodisk, Solna, Sweden) were placed on the plates which were incubated for 48 h in a microaerobic atmosphere (jars with GasPaks). Plates were read according to the manufacturer's recommendations.

The characteristics of the strains, cultured from biopsy specimens collected at inclusion, were studied. Strains were considered to be resistant to clarithromycin when the MIC was $\geq 1$ mg/liter.

\textbf{Determination of cagA status.} The cagA status was determined by PCR after DNA extraction. The biopsy samples were ground for 2 to 3 s with an electric tissue homogenizer and centrifuged for 5 min at 10,000 $\times$ g. The pellet was resuspended in 300 $\mu$L of extraction buffer (20 mM Tris-HCl pH 8; 0.5% Tween 20), and protease K (0.5 mg/mL) was added. The mixture was incubated for 1 h at 56°C. Finally, the enzyme was inactivated by boiling for 10 min.

The cagA status was determined by amplification of internal fragments of the \textit{cagA} gene, as described by Jenks et al. (16). Two sets of primers were used: the first one allowed the amplification of a 394-bp fragment (A1-A2), and the second allowed the amplification of a 717-bp fragment (A3-A4) (Table 1). The second amplification was performed only when the first was negative. A positive cagA status was defined as positive \textit{cagA} PCR results with one of the two primer sets.

\textbf{Statistical analysis.} The present analysis of the clinical trial database was performed in order to identify variables which were predictive or linked to the success or failure of \textit{H. pylori} eradication therapy, in particular, the cagA status of the strain.

A logistic regression model was constructed using information obtained at inclusion of the patients in the clinical trial as variables and the \textit{H. pylori} status evaluated at the end of the trial as the outcome measure. The variables used concerned (i) the host: age, gender, body mass index (BMI) (considered to be normal for women between 20.2 and 26.6 kg/m$^2$ and for men between 21.6 and 28.2 kg/m$^2$ and abnormal otherwise), ethnic origin (Caucasian or others, including Mediterranean, black, and Asian), tobacco smoking (yes or no), alcohol consumption (yes or no), compliance based on the number of pills brought back (good compliance when $>20\%$ of the pills were brought back); (ii) \textit{H. pylori} strain: the cagA status and susceptibility or resistance to clarithromycin as defined above; and (iii) the treatment received by the patient during the trial: pantoprazole o.d. versus pantoprazole b.i.d.

The EGRET statistical package (Statistic and Epidemiology Research Corporation, Seattle, Wash.) was used for univariate and multivariate analyses. All variables with a \textit{P} value of 0.25 or less in the univariate analysis were included in the full model (13). A backward elimination procedure was then performed to reduce the number of covariables (13). Only significant covariables (\textit{P} $\leq 0.05$) were retained in the models. The significance of the variables was tested using the likelihood ratio test. Confounding factors and interaction terms were taken into consideration as recommended (13). Estimated odds ratios (OR) and 95% confidence intervals (95% CI) were calculated from the coefficients.

\textbf{RESULTS}

\textbf{Sample.} Among 166 patients included in the PP analysis of the initial trial, 156 patients for whom the \textit{H. pylori} strains were available were included in the analysis of the present study. Strains from three patients could not be subcultured after initial culture, and in seven cases strains could not be recovered after thawing.

Among the 156 patients, 80 were male (51.3%), and the mean age was 51.7 years (range, 20 to 75 years; standard deviation, 14 years). The mean age of men and women was 49 and 54 years, respectively (\textit{P} = 0.38). The BMI ranged from 17.4 to 38.2 kg/m$^2$; the mean BMI among men was 25.4 and that among women was 24.8 (\textit{P} = 0.35). There were significantly fewer smokers among women (12 of 76 [15.8%]) than among men (25 of 80 [31.3%]) (\textit{P} = 0.02). Concerning the outcome, treatment was successful in 109 patients (69.9%).

Sixteen of 156 strains were clarithromycin resistant (10.2%). Seventy-four of 156 strains (47.4%) were cagA positive using the first set of primers. Among the remaining 82 strains negative for cagA, 10 (12.2%) were positive using the second set of primers; i.e., in total, 84 of 156 (53.8%) strains were cagA positive. There was no difference in the proportion of cagA-positive strains among the clarithromycin-resistant (8 of 16 [50%]) and -susceptible (76 of 140 [54.3%]) strains (\textit{P} = 0.73).

\textbf{Relationship between cagA status of strain and eradication outcome.} The univariate analysis (Table 2) showed that among the variable linked to eradication outcome at a sufficient level to be included in the regression model (\textit{P} $\leq 0.25$), two variables were associated with success—a double dose of pantoprazole versus a single dose and a BMI superior to the normal—and four variables were associated with failure: infection with a cagA-lacking strain, older age, tobacco smoking, and an ethnic origin other than Caucasian. \textit{H. pylori} was not successfully eradicated in any of the patients with clarithromycin-resistant strains. Among the other variables tested, such as gender and alcohol consumption, no association with eradication outcome was found.

\textbf{TABLE 2. Relationships between \textit{H. pylori} eradication failure and variables related to the host and the strains, in NUD patients (n = 156)$^a$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total sample</th>
<th>Eradication failure</th>
<th>OR for failure</th>
<th>95% CI</th>
<th>\textit{P}$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o.d.</td>
<td>80 51.3</td>
<td>32 40.0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b.i.d.</td>
<td>76 48.7</td>
<td>15 19.7</td>
<td>0.37</td>
<td>0.18–0.76</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>109 69.9</td>
<td>36 33.0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior to normal</td>
<td>20 12.8</td>
<td>6 30.0</td>
<td>0.87</td>
<td>0.31–2.45</td>
<td>0.791</td>
</tr>
<tr>
<td>Superior to normal</td>
<td>27 17.3</td>
<td>5 18.5</td>
<td>0.46</td>
<td>0.16–1.32</td>
<td>0.148</td>
</tr>
<tr>
<td>Susceptibility to clarithromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>140 89.7</td>
<td>31 22.1</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>16 10.3</td>
<td>16 78.0</td>
<td>NA$^d$</td>
<td>NA$^d$</td>
<td>NA$^d$</td>
</tr>
<tr>
<td>cagA status of strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cagA$^+$</td>
<td>84 53.8</td>
<td>20 23.8</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cagA-lacking</td>
<td>72 46.2</td>
<td>27 76.5</td>
<td>1.92</td>
<td>0.96–3.40</td>
<td>0.065</td>
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<tr>
<td>Ethnic origin of patient</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>134 86.5</td>
<td>38 28.4</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>21 13.5</td>
<td>9 42.9</td>
<td>0.89</td>
<td>0.66–5.38</td>
<td>0.18</td>
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<tr>
<td>Smoking status</td>
<td></td>
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<td></td>
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<tr>
<td>Nonsmoker</td>
<td>119 76.3</td>
<td>30 25.2</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>37 23.9</td>
<td>17 45.9</td>
<td>2.52</td>
<td>1.16–5.45</td>
<td>0.018</td>
</tr>
</tbody>
</table>

\textit{a} Results of univariate analysis.

\textit{b} \textit{P} not significant if $>0.25$ in univariate analysis.

\textit{d} Mean for total sample, 51.7 years; mean for eradication, failure, 48.5 years.

\textit{NA}, not applicable.
Because the resistance to clarithromycin predicted failure perfectly, the inclusion of this characteristic in the model was impossible. Therefore, in the regression model \((n = 156)\), two variables remained strongly associated with eradication failure—infection with a cagA-lacking strain \((OR = 2.2; 95\% CI, 1.1 \sim 4.7)\) and tobacco smoking \((OR = 3.1; 95\% CI, 1.3 \sim 7.0)\)—and one variable was associated with eradication success—a double dose of pantoprazole \((OR = 0.3; 95\% CI, 0.2 \sim 0.7)\) (Table 3). However, as the resistance characteristic of the strain was the main predictive factor of eradication treatment outcome, the same uni- and multivariate analyses were performed on susceptible strains only \((n = 140)\), showing the same results (Table 3).

**DISCUSSION**

Although the clinical trial was not initially designed for this type of analysis, the following results were forthcoming: in NUD patients, there is a clear relationship between eradication failure and the cagA status of the infecting strain (Table 3).

Patients with NUD constitute an interesting study population, because they have a highly heterogeneous distribution of cagA, and therefore the need for a large sample is alleviated. Indeed, in contrast to PUD patients, for whom the range of cagA-positive strains is from 80 to 90% in Western countries, the cagA gene is present in only 50 to 70% of the strains isolated from NUD patients (10, 17). In the present study, 53.8% of the strains were cagA positive. Furthermore, this study provides new information to help resolve the debate over whether to eradicate *H. pylori* in patients with NUD (5, 22, 27, 28).

The presence of the cagA gene was detected by PCR. The sensitivity of PCR is similar to that of colony hybridization when strains with negative results are tested with a second set of primers (17). Therefore, in this population comprised of NUD patients, the information on the cagA status of the strain is reliable and is a good predictive factor for eradication outcome.

From a biological point of view, the relationship between eradication outcome and cagA status can be explained by at least two different mechanisms. First, the presence of the cag pathogenicity island, as reliably detected by cagA (16, 20), induces the secretion of interleukin 8, a proinflammatory cytokine, by the epithelial cells, and an increased inflammation of the gastric mucosa in comparison to those harboring cagA-lacking strains is constantly found (7). The consequent increased blood flow may favor better diffusion of the antibiotics.

There are other examples of infectious diseases, such as meningitis and prostatis, for which this is the case. Another possible explanation may be related to the fact that cagA-positive strains grow faster than cagA-lacking strains. This point has not been extensively studied but is reported by two authors (7, 31). Mutants in the cag pathogenicity island obtained by Censini et al. exhibited a lower growth rate. Since antibiotics are active during cell division, they are more active on rapidly growing bacteria than on bacteria in stationary phase. Furthermore, the growth rate may influence the density, which is higher in the case of cagA-positive strains, and indirectly the inflammation (2, 12). The role of the cagA gene on eradication outcome may logically be explained by its effect on gastric mucosa. In any case, the cagA gene produces its effect on the outcome and the existing link is undisputable.

Infection with strains resistant to clarithromycin consistently led to treatment failure and therefore could not be included in the model. Indeed, a model cannot be adjusted on a variable for which there are no patients in one category (14), for example in this case, the category of resistant strains with eradication success. In order to avoid this problem inherent to the methodology, the analysis of cagA status alone was performed using both the entire sample and the subsample of patients harboring strains susceptible to clarithromycin only. The results were similar in both cases. As only 10% of the strains cultured were resistant, the results were valid whether or not these strains were included. Clarithromycin resistance was the strongest predictor of failure, and its global impact will be increasingly important as the prevalence rate of resistance augments. Amoxicillin resistance was not tested because no amoxicillin-resistant strains have yet been detected in France by the National Surveillance Network.

Finally, from a pragmatic viewpoint, it is possible to conclude that the cagA status of *H. pylori* is a good predictive factor for eradication outcome in NUD patients, independent of resistance status, at the present rate of clarithromycin resistance. This observation leads to two important recommendations. (i) In clinical practice, given the satisfactory correlation between the presence of the cagA gene in the strain and the serological detection of anti-CagA antibodies (9), when *H. pylori* eradication treatment is considered in NUD patients, it may be helpful in decision making to test for anti-CagA antibodies; a longer treatment may be necessary in CagA-negative patients. (ii) It should be mandatory that results of clinical trials in NUD patients be adjusted on the basis of cagA status of *H. pylori* strains.

**ACKNOWLEDGMENTS**

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**REFERENCES**


**TABLE 3. Results of multivariate analysis on variables associated with failure of eradication treatment of *H. pylori* infection**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Global sample ((n = 156))</th>
<th>Clarithromycin-susceptible strains (n = 140))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>cagA-lacking strain</td>
<td>2.2 1.1–4.7</td>
<td>2.6 1.1–4.7</td>
</tr>
<tr>
<td>Double dose of pantoprazole</td>
<td>0.3 0.2–0.7</td>
<td>0.3 0.1–0.7</td>
</tr>
<tr>
<td>Smoker</td>
<td>3.1 1.3–7.0</td>
<td>4.2 1.6–10.9</td>
</tr>
</tbody>
</table>

\(a\) Significance for each model, \(P < 0.001\).

\(b\) Susceptibility defined as MIC of <1 mg/liter.